New records of entomopathogenic fungi infecting Bemisia tabaci and Trialeurodes vaporariorum, pests of horticultural crops, in Argentina

Ana Clara Scorsetti · Richard A. Humber · Carolina De Gregorio · Claudia C. López Lastra

Received: 8 March 2007 / Accepted: 4 September 2007 / Published online: 2 October 2007 © International Organization for Biological Control (IOBC) 2007

Abstract The whiteflies Bemisia tabaci (Gennadius) and Trialeurodes vaporariorum (Westwood) (Hemiptera: Aleyrodidae) are major crop pests throughout the world. Although extensive research about biological control of whitefly by parasitoids and predators has been conducted, also entomopathogenic fungi can be considered as potential biological control agents. Surveys for entomopathogenic fungi were carried out in organic and conventional horticultural crops in greenhouses and open fields in Buenos Aires and Corrientes provinces, Argentina. These surveys resulted in the recovery and isolation of the following fungi from whiteflies: Lecanicillium lecanii (Zimmerm.) Zare & W. Gams, L. muscarium (Petch) Zare & W. Gams, L. longisporum (Petch) Zare & W. Gams, Isaria fumosorosea Wize and I. javanica (Frieder. & Bally) Samson & Hywel-Jones. Pathogenicity tests were conducted against T. vaporariorum nymphs using a conidial suspension $(1 \times 10^7 \text{ conidia/ml})$ of the fungi. A mortality rate between 26.6% and 76.6% was obtained at 7 days post-infection. These are the first records of natural infections in the southernmost region of the South American continent of L. lecanii, L. muscarium, L. longisporum and Isaria javanica (Ascomycota: Hypocreales) on T. vaporariorum and also the first report of I. fumosorosea on B. tabaci.

Keywords Aleyrodidae · Ascomycota · Biological control · Fungal entomopathogens · Horticultural crops · Hypocreales · Isaria spp. · Lecanicillium spp. · Paecilomyces spp. · Verticillium spp. · Whiteflies

e-mail: ascorsetti@cepave.edu.ar

R. A. Humber

Plant, Soil and Nutrition Laboratory, USDA-ARS, Tower Road, Ithaca, NY 14853, USA



A. C. Scorsetti (☒) · C. De Gregorio · C. C. López Lastra CEPAVE (Centro de Estudios Parasitológicos y de Vectores) UNLP-CONICET, Calle 2 #584, La Plata 1900, Argentina

Introduction

Whiteflies (Hemiptera: Aleyrodidae) such as the sweetpotato whitefly *Bemisia tabaci* (Gennadius) and the greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) are major insect pests in the world due to the role as vectors of plant viruses like the "geminivirus" (Brown 1998; Faria and Wraight 2001). *Bemisia argentifolii* Bellows & Perring (*Bemisia tabaci* strain B) is a highly polyphagous species that feeds on, among others, cotton, cucurbits, lettuce, soybean and tomatoes and its pest status has increased strongly since the 1980s (Osborne 1988; Hoelmer et al. 1991). *T. vaporariorum* is also a pest of horticultural crops and many others ornamental plants, especially in greenhouses, and has been reported to feed on about 250 species of plants around the world (Landa et al. 1994). Thirteen species of whiteflies have been reported in Argentina and of these species, only *Aleurothrixus aepim* (Goldi), *Aleurothrixus floccosus* (Maskell), *B. tabaci*, *Siphoninus phillyreae* and *T. vaporariorum* causes economic damage to crops and trees (Viscarret 2000).

Pesticide resistant whitefly populations have emerged as a consequence of intensive use of chemical insecticides (Faria and Wraight 2001). Pesticide resistance in whiteflies has stimulated studies on integrated pest management, in which biological control may play a significant role. Although extensive research about biological control of whitefly has been conducted on parasitoids and predators, also entomopathogenic fungi can be considered as potential biological control agents. Entomopathogenic fungi of whitefly are able to invade the insect actively through the cuticle, which is an advantage for the management of piercing—sucking insects and show a particular promise for whitefly control (Lacey et al. 1995).

More than 20 different species of fungi have been reported worldwide to infect whiteflies. These include Aschersonia aleyrodis Webber, A. andropogonis P. Henn, A. cf. goldiana Sacc. & Ellis, Beauveria bassiana (Balsamo) Vuillemin, Isaria fumosorosea Wize (as Paecilomyces fumosoroseus), Lecanicillium lecanii (Zimmerm.) Zare & W. Gams (as Verticillium lecanii), Conidiobolus spp., Entomophthora sp., Zoophthora radicans (Brefeld) Batko (Faria and Wraight 2001), and Isaria farinosa (Holmsk.) Fr. (as Paecilomyces farinosus) (Landa et al. 1994).

The fungus *I. fumosorosea* Wize is one of the most promising fungal species for control of *B. argentifolii* (Vidal et al. 1998) and *L. lecanii* has been used as a biological control agent against *T. vaporariorum* (Cuthbertson et al. 2005). Furthermore, several isolates of *B. bassiana* and *L. muscarium* have been selected as mycoinsecticides for whitefly control (Faria and Wraight 2001).

In South America reports of entomopathogenic fungi affecting whiteflies are scarce (Drummond et al. 1987; Sosa Gomez et al. 1997; Gomez 1999; Lourençao et al. 1999). In Argentina, *L. lecanii* was reported previously by López Lastra (1989) and Yasem de Romero (1992) from Coleoptera and Hemiptera, respectively. *Isaria fumosorosea* was the only fungal species that has been recorded infecting *T. vaporariorum* in tomato crops in Buenos Aires province, Argentina (Toledo et al. 2004).

The objectives of this study were to search for native fungal pathogens of whiteflies under natural field conditions, to characterize those fungal isolates and to evaluate their pathogenicity for the whitefly *T. vaporariorum*. This paper provides the first record of natural infections of entomopathogenic fungi (Ascomycota: Sordariomycetes: Hypocreales) on *B. tabaci* strain B, in the southernmost region of the South American continent. The paper also presents new knowledge of fungal pathogens that are able to infect *T. vaporariorum*.



Materials and methods

Collection of insects

A survey for whiteflies was carried out in organic and conventional horticultural crops in greenhouses and open fields in Buenos Aires and Corrientes provinces, Argentina (Fig. 1). A total of six locations were sampled: Colonia Urquiza (La Plata county) 34°56'19.2" S/58°06'3.8" W; Parque Pereyra Iraola (Berazategui county, Buenos Aires province) 34°50'36.5" S/58°05'33.2" W; El Peligro (La Plata, county Buenos Aires province) 34°56' S/58°10' W; Arana (La Plata county, Buenos Aires province) 35°0'19.8"

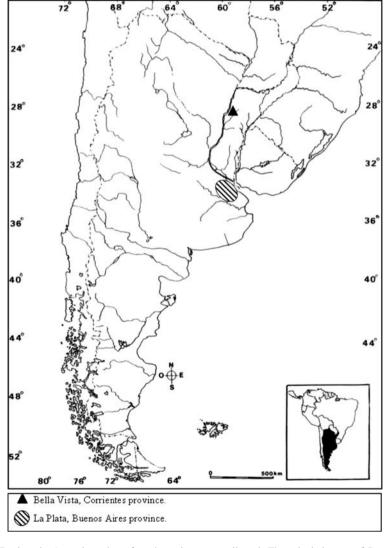


Fig. 1 Regions in Argentina where fungal species were collected. These include part of Buenos Aires Province and Corrientes Province



S/57°55'30" W; General Mansilla (Magdalena county, Buenos Aires province) 35°05'30.7" S/57°45'20.6" W and Monte Caseros (Corrientes province) 30°15'04" S/57°39'2.2" W. Whiteflies were collected from the following horticultural crop hosts: Apium graveolens L. (celery), Beta vulgaris var. cicla L. (Swiss chart), Beta vulgaris var. conditiva L. (red beet), Capsicum annuum L. (pepper), Cucumis sativus L. (cucumber), Cynara scolymus L. (artichoke), Lactuca sativa L. (lettuce), Lycopersicon esculentum Mill. (tomato) and Solanum melongena L. (eggplant). Surveys were conducted over a 4-year period (Jan 2002-Dec 2005) at weekly intervals. Twenty plants of each species were randomly selected using a hand-calculator, and checked each week at each of the six locations. To collect immature instars, two leaves were selected from the upper, middle and bottom levels of the canopy of each plant. Of leafy vegetables the whole plant was checked. Adult insects were sampled from the leaves in the two upper levels of the canopy. Living whiteflies were caught and placed individually in sterilized plastic cups (150 cm³) with lids. Dead insects with or without external signs of mycosis were collected with the piece of plant material on which they were collected in sterilized individual plastic containers (100 cm³). All collected material was transported to the laboratory for analysis of fungal infection. Dead whiteflies without external fungal signs of mycoses were put in 60-mm Petri dishes with a dampened filter paper and maintained at 20°C for 24-72 h to allow development of any fungi present in them.

Identification of fungal pathogens

Infected whiteflies, covered by ashy-cottony mycelia, were examined under a Zeiss DV4 dissecting stereomicroscope. Mycelia were mounted in lactophenol/cotton blue (0.01% w/v) and observed by phase contrast with an Olympus CH3 microscope. Fungal preparations were photographed using a Nikon Optiphot microscope equipped with differential interference contrast (DIC) fitted with a Canon PowerShot A80 camera. Measurements of length and width of fungal structures (conidia, conidiogenous cells and mycelia) from fresh infected cadavers were made to enable specific identification. Fungal species were identified according to taxonomic keys and monographs in Samson (1974), Samson et al. (1988) and Zare and Gams (2001).

Isolation and description of fungal pathogens

Monosporic isolates were obtained from infected whiteflies as described by Lecuona (1996), and inoculated on 2% malt extract agar (MEA) with 40,000 units/ml penicillin G (Merck[®], Germany) and 80,000 units/ml streptomycin (Parafarm[®], Argentina). Microscopic and macroscopic descriptions were made from cultures growing on MEA according to the standards used by Samson (1974) and Zare and Gams (2001). Fungal isolates were deposited in the Mycological Collections at the Centro de Estudios Parasitológicos y de Vectores (CEP, La Plata, Argentina) and at the USDA-ARS Collection of Entomopathogenic Fungal Cultures (ARSEF, Ithaca, New York).

Pathogenicity assays

The pathogenicity of fungal isolates was tested on T. vaporariorum nymphs. Insects were reared on bean plants ($Phaseolus\ vulgaris\ L$.) and maintained in cages ($1\ m \times 1\ m \times 1\ m$)



under greenhouse conditions at 20 ± 2°C and 16:8 h light:dark (L:D). Conidial viability of each isolate was assessed after 24 h using the techniques described by Lane et al. (1988). For each isolate, 25 fourth-instar nymphs were removed from the bean leaves. They were sprayed by using a glass nozzle of 35 cc capacity with 300 µl of a conidial suspension of 1×10^7 conidia/ml in 0.01% (v/v) Tween 80 (sodium polysorbate) using always re-isolated fungus for each fungal species. Controls were sprayed with only 300 µl of 0.01% Tween 80. Three replicates and a control were performed for each isolate. Afterwards, nymphs were arranged over a disc of bean leaf (30 mm diameter) and kept in cages (35 mm diam) with sterilized filter paper at the bottom to maintain 95% relative humidity. Treated and control insects were held at 24 ± 2°C, 70% RH and a photoperiod of 14:10 h (light:dark). Cumulative mortality determined by the presence of mycelial mass (Landa et al. 1994) was recorded daily for 7 days. Dead insects were removed daily and superficially sterilized by placing the specimens in 70% ethanol for a few seconds, then washing them in sterile distilled water, followed by 0.5% sodium hypochlorite for 1 min, and rinsed again in distilled water according to Lacey and Brooks (1997). Next they were placed in Petri dishes with filter paper moistened with sterile distilled water and incubated at 25°C for 3-5 days to allow fungal development. Fungal infections were confirmed with light microscopy for all dead insects, and they were mounted in lactophenol/cotton blue to check for fungal infection.

Mortality in the tests was corrected by the mortality found in the controls according to Abbott (1925). Differences between mortality of replicates were analyzed using ANOVA (Statistica 1998). The median lethal time was obtained according to methodology cited by Lecuona and Díaz (2001).

Results

The following five fungal species were identified and isolated from whiteflies of horticultural crops, mainly from greenhouses: *L. lecanii*, *L. muscarium*, *L. longisporum*, *I. fumosorosea*, and *I. javanica*. Infected whiteflies were always found at the underside of the leaves and were covered by white cottony mycelia.

Descriptions of fungal pathogens

Lecanicillium lecanii (Zimmerm.) Zare & W. Gams, Nova Hedwigia 72:333, 2001.

- ≡ Cephalosporium lecanii Zimmermann. Teysmania 9:243, 1899. (basionym)
- = Verticillium lecanii (Zimmerm.) Viégas, Rev. Inst. Café São Paulo 14:754, 1939.

Hosts: adults of T. vaporariorum on A. graveolans, in greenhouse. Locality: El Peligro

Colony diameters reached 29 mm in 10 days on MEA at 24°C in darkness, rather compact, white, with clear yellow on reverse. Phialides relatively short, (13.9) 21.8 ± 3.9 (30.7) × (0.9) 1.15 ± 0.3 (1.9) µm (n = 25), conical, aculeate, produced singly or in whorls of up to six. Conidia hyaline, short ellipsoidal, (1.9) 3.5 ± 0.9 (5.4) × (0.8) 1 ± 0.2 (1.5) µm (n = 70), formed in heads at the apex of the phialides. One isolate in pure culture was established on MEA. Conidial viability was 100% at 24 h. The mortality of *T. vaporariorum* nymphs exposed to a conidial suspension was $52.6 \pm 8.3\%$ at 7 days post-treatment; the median lethal time was 4.32 days (Table 1).



	e , ,	1	, e
Fungus species	Number of isolate	Fungus collected on	Percent mortality (means ± SD) on <i>T. vaporariorum</i>
L. lecanii	CEP 057 = ARSEF 7207	T. vaporariorum	52.6 ± 8.3
L. longisporum	CEP $056 = ARSEF 7461$	T. vaporariorum	35.0 ± 5.0
L. muscarium	CEP 054 = ARSEF 7460	T. vaporariorum	65.0 ± 23.0
Isaria fumosorosea	CEP 206	Bemisia tabaci strain B	76.7 ± 3.0
I. javanica	CEP 107 = ARSEF 7477	T. vaporariorum	26.6 ± 10.4

Table 1 Fungal isolates obtained from whiteflies sampled on horticultural crops in Buenos Aires and Corrientes provinces (Argentina) and mortality of *T. vaporariorum* after 7 days caused by each fungus

Lecanicillium longisporum (Petch) Zare & W. Gams

- ≡ Cephalosporium longisporum Petch, Trans. Brit. Mycol. Soc. 10:166, 1925. (basionym)
- = Acrostalagmus aphidum Oudem., Nederl. Kruidk. Arch. 3:759, 1902.
- = Cephalosporium dipterigenum Petch, Naturalist 1931:102.

Hosts: adults of T. vaporariorum on A. graveolans, in greenhouse. Locality: El Peligro

Colonies grew moderately slow on MEA, attaining a diameter of about 8.4 (\pm 1.2) mm after 10 days at 24°C in darkness, consisting of a homogeneous diameter and regular borders, white and smooth mycelia, with deep yellow reverse. Vegetative hyphae hyaline (3.5) 4.3 \pm 0.6 (4.7) μ m wide (n = 25). Conidiophores erect, septate, (2.1) 2.4 \pm 0.1 (2.8) μ m wide (n = 25). Phialides tapering towards the apice, subaculeate (17.3) 20.2 \pm 1.9 (24.7) \times (0.7) 1 \pm 0.2 (1.5) μ m (n = 35), singly or in whorls up to 3, rarely 4, at each node. Conidia produced in globose heads, hyaline, ellipsoidal to oval (3) 4.4 \pm 1.3 (8) \times (0.9) 1.2 \pm 0.2 (1.5) μ m, rarely 1-septate (n = 80). Two isolates in pure cultures were established on MEA. Conidial viability was 100% at 24 h for both isolates. The mortality of T. t0.2 t1 t2 t3 t4 t4 t5 t5% at 7 days post-treatment (Table 1).

Lecanicillium muscarium (Petch) Zare & W. Gams

- ≡ Cephalosporium muscarium Petch, Naturalist 102, 1931. (basionym)
- = Oospora necans Saccardo & Trotter, Ann. Mycol. 3:514, 1905.
- = Cephalosporium lefroyi Horne, Gard. Chron. 57: 139, 1915; Trans. Brit. Mycol. Soc. 5:241, 1915.
- = Cephalosporium aphidicola Petch, Trans. Brit. Mycol. Soc. 16:71, 1931.
- = Cephalosporium thripidum Petch, Trans. Brit. Mycol. Soc. 16:234, 1932.
- = Cephalosporium subclavatum Petch, Trans. Brit. Mycol. Soc. 25:262, 1942.

Hosts: adults of T. vaporariorum, on A. graveolans, in greenhouse. Locality: El Peligro

Colonies on MEA grew moderately fast, attaining 28.3 (± 0.6) mm diameter after 10 days at 24°C in darkness, consisting of homogeneous diameter and regular borders, white and smooth mycelia, with deep yellow reverse. Phialides longer than those of *L. lecanii*, hyaline, conical, singly or in whorls up to five in each node, (18.9) 22.3 \pm 3.7 (28.7) × (0.8) 1.1 \pm 0.1 (1.3) μ m (n = 25). Conidia hyaline, ellipsoidal and irregular in size and shape, produced in globose heads, (3.9) 4.8 \pm 0.7 (6.1) × (0.9) 1.1 \pm 0.2 (1.8) μ m



(n = 60). One isolate in pure culture was established on MEA. Conidial viability was 95.3% (±1.8) at 24 h. The mortality of *T. vaporariorum* nymphs exposed to a conidial suspension was 65 ± 22.9% at 7 days post-treatment, the median lethal time was 4.35 days (Table 1).

Isaria fumosorosea Wize

- ≡ *Isaria fumosorosea* Wize, Bull. Int. Acad. Pol. Sci. Lett., 72, 1904.
- ≡ Spicaria fumosorosea (Wize) Vassiljevski, Morbi Plant.:146, 1921.
- ≡ Paecilomyces fumosoroseus (Wize) A.H.S. Brown & G. Smith, Trans. Br. Mycol. Soc. 40:67, 1957.

Hosts: nymphs and adults of *B. tabaci* strain B, on *C. annuum* and *C. sativus* in greenhouses. Locality: Monte Caseros

Colonies grew moderately fast on MEA, attaining 29 ± 0.6 mm diameter after 10 days at 24°C in darkness, consisting of a basal felt with raised floccose overgrowth, homogeneous diameter and regular borders, reverse initially colourless but later becoming pink. Vegetative hyphae smooth walled, hyaline, $2.5 \pm 0.5 \,\mu\text{m}$ wide (n = 25); conidiophores $2.4 \pm 0.5 \,\mu\text{m}$ wide (n = 25), smooth walled, consisting of verticillate branches bearing whorls of 4–6 phialides. Phialides with a globose basal portion tapering into a long distal neck (4.9) $6.3 \pm 0.9 \, (7.9) \times (1.1) \, 1.8 \pm 0.3 \, (2.5) \,\mu\text{m}$ (n = 25). Conidia hyaline to slightly pink, cylindrical to fusiform (3.5) $4.6 \pm 0.5 \, (5.4) \times (0.9) \, 1.2 \pm 0.2 \, (1.6) \,\mu\text{m}$ (n = 80). Eight isolates in pure cultures were established on MEA (Table 1). Conidial viabilities were 100% for CEP 203, 100% for CEP 204, 99.5 ± 1 % for CEP 205, 100% for CEP 206, 100% for CEP 207, 98.7 ± 1.3 % for CEP 208, 96.3 ± 3.2 % for CEP 209, and 99.3 ± 1.7 % for CEP 210 after 24 h. The mortality of T. vaporariorum nymphs exposed to a conidial suspension of I. fumosorosea CEP 206 was 76.7 ± 2.9 % at 7 days post-treatment; the median lethal time was 4.5 days (Table 1).

Isaria javanica (Frieder. & Bally) Samson & Hywel-Jones, Mycol. Res. 109, 588, 2005.

- ≡ *Spicaria javanica* Friederichs & Bally, Meded. Koffiebessenboeboek-Fonds 6:146, 1923. (basionym)
- ≡ Paecilomyces javanicus (Friedrichs & Bally) A.H.S. Brown & G. Smith, Trans. Brit. Mycol. Soc. 40, 65, 1957
- = Spicaria formosensis Sawada, Spec. Publ. Coll. Agric. Natn. Taiwán Univ. 8, 188, 1959.

Hosts: adults of *T. vaporariorum* on tomato crops in greenhouse. Locality: Colonia Urquiza

Colonies grew moderately fast on MEA, attaining a diameter of about 29.6 (±0.6) mm after 10 days at 24°C in darkness, consisting of a basal felt with raised floccose overgrowth, producing a few synnemata, homogeneous diameter and regular borders, white at first becoming cream-colored. Reverse uncoloured to yellow.

Vegetative hyphae smooth walled, septate, hyaline, $1.2 \pm 0.2 \, \mu m$ wide (n = 25). Conidiophores smooth walled, consisting of verticillate branches bearing whorls of 2–3 phialides. Phialides with a globose basal portion tapering into a long distal neck (4.9) $6.5 \pm 1.1 \, (7.9) \times (0.9) \, 1.6 \pm 0.3 \, (2.5) \, \mu m \, (n = 25)$. Conidia hyaline, cylindrical to fusiform (2.5) $4.3 \pm 0.5 \, (5.9) \times (0.9) \, 1.6 \pm 0.3 \, (2) \, \mu m \, (n = 70)$. One isolate in pure culture was established on MEA. Conidial viability was $98.8\% \pm 0.2\%$ at 24 h. The mortality of *T. vaporariorum* nymphs exposed to a conidial suspension was $26.6 \pm 10.4\%$ at 7 days post-treatment (Table 1).



Statistical analysis of fungal pathogenicity

All fungal species tested were pathogenic to the whiteflies at conidial concentrations of 1×10^7 conidia/ml. Seven days after inoculation, there were no significantly differences between replicates (F = 3.78, df = 5; P = 0.216).

Discussion

The most common fungal pathogens of *Bemisia* spp. and other whiteflies are *Paecilomyces fumosoroseus*, *Verticillium lecanii* and *Aschersonia* spp. (Humber 1992; Lacey et al. 1993, 1995). Very few records of entomopathogenic fungi of whiteflies are known for the Neotropic region (Gomez 1999; Faria and Wraight 2001; Toledo et al. 2004). We found five fungal species that infected whiteflies on horticultural crops at six locations in Buenos Aires and Corrientes provinces (Argentina) during 4-year survey. Characterization of the fungal isolates did not show major differences in morphology or growth in comparison with earlier descriptions of these species (Samson 1974; Zare and Gams 2001).

In our research, *Isaria fumosorosea* was the most frequently collected fungus on whiteflies. Another species of this genus, *Isaria javanica* (= *Paecilomyces javanicus*), was previously recorded as pathogen of Lepidoptera (Samson 1974) and later of Coleoptera and whiteflies (*Bemisia* sp., Hemiptera) (ARSEF website: http://arsef.fpsnl.cornell.edu). Here, we report the first record of *I. javanica* for South America.

Lecanicillium lecanii is a common fungal pathogen of aphids, scales, whiteflies and several other insects (Evans and Samson 1986). In Argentina, L. lecanii was cited previously by López Lastra (1989) and by Yasem de Romero (1992), infecting Coleoptera and Coccidea, respectively. L. muscarium is an insect pathogen that is used commercially to control greenhouse pests and has been shown to be a candidate species for the control of B. tabaci (Cuthbertson et al. 2005). In Argentina this fungal species has been isolated from Delphacodes kuscheli (Hemiptera: Delphacidae) (Toledo et al. 2007). The related species L. longisporum was isolated from adults of T. vaporariorum only once during this survey in La Plata, Argentina. Our discovery of the entomopathogenic fungi L. muscarium and L. longisporum represents the first records for South America, and expands both their geographical distribution and host range.

After isolation and characterization of the fungal species, we tested their pathogenicity with regard to *T. vaporariorum*. Though the conidial viabilities obtained from the fungal species were always above 90%, we found a high variation in insect mortality, ranging from 26 to 76% (Table 1). Lacey et al. (1995) reported distinct differences in the apparent host specificity of different strains of *V. lecanii* isolated from aphids, whiteflies and other insects, although the strains isolated from whiteflies were more active and virulent against this target host. They also observed that treated eggs were not infected, but that nymphs that hatched from these eggs were later infected at the same rate and to the same degree as first instar nymphs treated directly after hatching. In our study we observed that adults emerging from treated pupae could also die later and to present symptoms of fungal infection by the fungi *I. fumosorosea*, *I. javanica* and *L. muscarium*.

Lacey (1999) reported mortality of eggs and second-instar nymphs of *B. tabaci* exposed to two different strains of *P. fumosoroseus*. Mortalities at 14 days post treatment ranged from 8.3–14.2% for eggs and 26.6–59.1% for nymphs treated with a conidial suspension of 1×10^8 conidia/ml and 2×10^7 for eggs and nymphs, respectively. The results of this study show a high nymphal mortality by *I. fumosorosea* (76.7 \pm 3% at 7 days post-treatment).



Cuthbertson et al. (2005) tested *L. muscarium* from the commercial Mycotal formulation (Koppert Biological Systems) against immature stages of *B. tabaci* on tomato and verbena plants. They reported that first and second instar nymphs were more susceptible than third or fourth instars, with 50%, 55%, 25% and 15% mortality from first to fourth instars, respectively. In the present study, mortality of *T. vaporariorum* caused by *L. muscarium* (65 \pm 23%) was variable, but higher than that found by Cuthbertson et al. (2005).

The results of our study show that local collection of entomopathogenic fungi in the southern most part of South America offers excellent possibilities to find candidates for biological control of whiteflies. Some of the isolated fungus species showed a high mortality of greenhouse whitefly, one of the most problematic whitefly species worldwide.

Acknowledgments The authors are indebted to and gratefully thank Ing. S. Cáceres for the contribution of infected insects from Corrientes province, Dra. C. Cédola for collaborating with the identification of the insects, and Joop van Lenteren for critical review of the manuscript.

References

Abbot WS (1925) A method for computing effectiveness of an insecticide. J Entomol 18:265-267

Brown JK (1998) Diversity and global distribution of whitefly-transmitted geminiviruses of cotton. Arizona Agric Exp Stn 112:587–598

Cuthbertson AGS, Walters KFA, Northing P (2005) The susceptibility of immature stages of *Bemisia tabaci* to the entomopathogenic fungus *Lecanicillium muscarium* on tomato and verbena foliage. Mycopathologia 159:23–29

Drummond J, Heale JB, Gillespie AT (1987) Germination and effect of reduced humidity on expression of pathogenicity in *Verticillium lecanii* against the glasshouse whitefly *Trialeurodes*. Ann Appl Biol 111:193–201

Evans HC, Samson RA (1986) The genus *Verticillium*: toxonomic problems in species with invertebrate hosts. Proceedings of the IV International Colloquium of Invertebrate Pathology, pp 86–189

Faria M, Wraight SP (2001) Biological control of *Bemisia tabaci* with fungi. Crop Protect 20:767–778

Gomez H (1999) Aislamiento e identificación de hongos entomopatógenos de la "mosca blanca" Bemisia tabaci (Gennadius) (Homoptera: Aleyrodidae) en Lima, Perú. Rev Per Ent 41:83–86

Hoelmer KA, Osborne LS, Yokomi RK (1991) Foliage disorders in Florida associated with feeding by sweetpotato whitefly, *Bemisia tabaci*. Florida Entomol 74:162–166

Humber RA (1992) Collection of entomopathogenic fungal cultures, USDA, ARS-110, 177 pp

Lacey LA (1999) Ovicidal and larvicidal activity of conidia and blastospores of *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes) against *Bemisia argentifolii* (Homoptera: Aleyrodidae) with a description of a bioassay system allowing prolonged survival of control insects. Biocontrol Sci Techn 9:9

Lacey L, Brooks WM (1997) Initial handling and diagnosis of diseased insects. In: Lacey LA (ed) Manual of techniques in insect pathology. Academic Press, San Diego, California

Lacey LA, Kirk AA, Hennessey RD (1993) Foreign exploration for natural enemies of *Bemisia tabaci* and implementation in integrated control programs in the United States. Proceedings Association Nationale de Protection des Plantes 3rd International Conference on Pest in Agriculture, vol 1, pp 351–360

Lacey LA, Fransen JJ, Carruthers R (1995) Global distribution of naturally occurring fungi of *Bemisia*, their biologies and use as biological control agents. In: Gerling D, Andover H (eds) *Bemisia* 1995: taxonomy, biology, damage, control and management. Intercept Ltd, UK, pp 401–433

Landa Z, Osborne LS, Lopez F, Eyal J (1994) A bioassay for determining pathogenicity of entomogenous fungi on whiteflies. Biol Control 4:341–350

Lane BS, Humphreys AM, Thompson K, Trinci APJ (1988) ATP Content of stored spores of *Paecilomyces farinosus* and the use of ATP as criterion of spore viability. Trans Br Mycol Soc 90:109–111

Lecuona RE (ed) (1996) Microorganismos Patógenos Empleados en el Control Microbiano de Insectos Plaga. Talleres gráficos Mariano Mas, Buenos Aires, Argentina

Lecuona RE, Díaz BM (2001) Susceptibilidad de Spodoptera frugiperda (J.E. Smith) a los hongos entomopatógenos Nomuraea rileyi, Metarhizium anisopliae y Beauveria bassiana. RIA (Revista de Investigaciones Científicas) 30:25



López Lastra CC (1989) Nuevos hospedadores para los hongos entomopatógenos *Metarhizium anisopliae* (Metchnikoff) Sorokin y *Verticillium lecanii* (Zimmerman) Viegas, en la República Argentina. Rev Soc Ent Arg 47:94

- Lourençao AL, Yuki VA, Alves SB (1999) Epizootia de *Aschersonia* cf. *goldiana* em *Bemisia tabaci* (Homoptera: Aleyrodidae) biotipo B no Estado de Sao Paulo. An Soc Entomol Brasil 28:343–345
- Osborne LS (1988) The not so sweet potato whitefly. Florida Foliage 14:8-15
- Samson RA (ed) (1974) Paecilomyces and some allied Hyphomycetes. Studies in Mycology, vol 6. Baarn, The Netherlands
- Samson RA, Evans HC, Latgé JP (eds) (1988) Atlas of entomopathogenic fungi. Springer-Verlag, Berlin Sosa Gómez DR, Moscardi F, Santos M (1997) Bemisia spp. na cultura da soja: ocorrência, controle químico e incidência de fungo entomopatógenico Paecilomyces spp. In: 16th Congresso Brasileiro de Entomologia, Resumos. SEB/Embrapa-CNPMF, Salvador, Brazil, p 144
- Statistica for Windows, StatSoft Inc (1998) Computer program manual. StatSoft, Inc, Tulsa, OK
- Toledo AV, Scorsetti AC, Dikgolz VE, López Lastra CC (2004) *Paecilomyces fumosoroseus y Nomuraea rileyi* (Deuteromycotina: Hyphomycetes), hongos patógenos de insectos plaga de la agricultura en la Argentina. Bol Soc Argent Bot 39:21–26
- Toledo AV, Remes Lenicov AM, López Lastra CC (2007) Pathogenicity of fungal isolates (Ascomycota: Hypocreales) against *Peregrinus maidis*, *Delphacodes kuscheli* (Hemiptera: Delphacidae), and *Dalbulus maidis* (Hemiptera: Cicadellidae), vectors of corn disease. Mycopathologia 163(4):225–232
- Vidal C, Osborne LS, Lacey LA, Fargues J (1998) Effect of host plant on the potencial of *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes) for controlling the silverleaf whitefly, *Bemisia argentifolii* (Homoptera: Aleyrodidae) in greenhouses. Biol Control 12:191–199
- Viscarret MM (2000) Estudios biológicos sobre Aleyrodidae (Insecta: Hemiptera) con especial énfasis en el complejo *Bemisia tabaci* y su posible control biológico. Doctoral Thesis, Facultad de Ciencias Exactas y Naturales, Univ. Buenos Aires
- Yasem de Romero MG (1992) Estudio comparativo del entomopatógeno *Verticillium lecanii* (Zimm.) Viegas de distinta procedencia. Rev Agron NO Argent 26:71–87
- Zare R, Gams W (2001) A revision of *Verticillium* sect. *Prostata* IV. The genera *Lecanicillium* and *Simplicillium* gen. nov. Nova Hedwigia 73:1–50

